

# The use of 1 $\alpha$ ,25-dihydroxyvitamin D3 as an anticancer agent

Brown, Geoffrey; Wallace, Graham; Marcinkowska, Ewa

DOI:  
[10.3390/ijms17050729](https://doi.org/10.3390/ijms17050729)

License:  
Creative Commons: Attribution (CC BY)

*Document Version*  
Publisher's PDF, also known as Version of record

*Citation for published version (Harvard):*  
Brown, G, Wallace, G & Marcinkowska, E 2016, 'The use of 1 $\alpha$ ,25-dihydroxyvitamin D3 as an anticancer agent', *International Journal of Molecular Sciences*, vol. 17, no. 5, 729. <https://doi.org/10.3390/ijms17050729>

[Link to publication on Research at Birmingham portal](#)

## General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

## Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact [UBIRA@lists.bham.ac.uk](mailto:UBIRA@lists.bham.ac.uk) providing details and we will remove access to the work immediately and investigate.



Review

# The use of $1\alpha,25$ -dihydroxyvitamin $D_3$ as an anticancer agent

Ewa Marcinkowska<sup>1</sup>, Graham R. Wallace<sup>2</sup> and Geoffrey Brown<sup>3\*</sup>

<sup>1</sup> Laboratory of Protein Biochemistry, Faculty of Biotechnology, University of Wrocław, Joliot-Curie 14a, 50-383 Wrocław, Poland; ema@cs.uni.wroc.pl

<sup>2</sup> Institute of Inflammation and Aging, College of Medical and Dental Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK; g.r.wallace@bham.ac.uk

<sup>3</sup> Institute of Clinical Sciences, College of Medical and Dental Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK; g.brown@bham.ac.uk

\* Correspondence: g.brown@bham.ac.uk; Tel.: +0121 414 4082

Academic Editor: name

Received: date; Accepted: date; Published: date

**Abstract:** The notion that vitamin D can influence the incidence of cancer arose from epidemiological studies. The major source of vitamin D in the organism is skin production upon exposure to ultra violet-B. The very first observation of an inverse correlation between exposure of individuals to the sun and the likely hood of cancer was reported as early as 1941 [1]. In 1980, Garland and Garland hypothesised from findings from epidemiological studies of patients in the US with colon cancer that vitamin D produced in response to sun exposure is protective against cancer as opposed to sunlight *per se* [2]. Later studies revealed inverse correlations between sun exposure and the occurrence of prostate [3] and breast [4] cancers. These observations prompted laboratory investigation of whether or not vitamin D had an effect on cancer cells. Vitamin D is not active against cancer cells, but the most active metabolite  $1\alpha,25$ -dihydroxyvitamin  $D_3$  (1,25D) has profound biological effects. Here we review the anticancer action of 1,25D, clinical trials of 1,25D to date and the prospects of the future therapeutic use of new and low calcaemic analogues.

**Keywords:** cancer;  $1\alpha,25$ -dihydroxyvitamin D; analogues

## 1. Introduction

Differentiation of mouse myeloid leukemia cell line towards macrophage-like cells in response to a sub-nanomolar concentration of  $1\alpha,25$ -dihydroxyvitamin  $D_3$  (1,25D) was reported in 1981 [5]. The changes observed were striking; the leukemic cells exposed to 1,25D not only looked different, but were also able to phagocytose, produce antibacterial agents, migrate and adhere to substratum [5]. A similar effect of 1,25D on human acute myeloid leukemia (AML) cells was reported soon after [6]. In this case, changes in cell cycle progression were observed to accompany differentiation to monocytes. The changes were a transient increase in the proportion of cells in the  $G_2/M$  phase of cell cycle, followed by an arrest in the  $G_1/G_0$  phase [7]. In other words, 1,25D provides an initial signal for cell proliferation, followed by different signal leading to cell maturation and cycle arrest [8]. This complicated set of events, which is triggered by 1,25D, requires the coordinated actions of signalling molecules and transcription factors.

## 2. $1\alpha,25$ -Dihydroxyvitamin $D_3$ -driven cell signalling in AML cells

It's widely accepted that expression of a functional vitamin D receptor (VDR) within AML cells is required for 1,25D to exert its biological effects [9]. In support of this viewpoint is that AML cells which express VDR at a low level are resistant to the differentiating effects of 1,25D, and the

sensitivity of AML cells to 1,25D is restored when the level of VDR is upregulated [10]. That the presence of VDR is essential to the differentiating action of 1,25D was confirmed by the finding that normal blood progenitor cells from VDR knockout mice failed to differentiate into monocytes in response to 1,25D [11].

VDR is a ligand-activated transcription factor that controls the transcription of hundreds of genes, either directly or indirectly [12]. Among the VDR-target genes are ones that encode the functional attributes of mature macrophages, such as cluster of differentiation (CD) 14 [13], and genes that control cell cycle progression are 1,25D responsive [14]. VDR protein is translated in the cytosol, and its site of transcriptional activity is the cell nucleus. Therefore, efficient nuclear translocation of VDR is prerequisite for its activity and AML cells in which nuclear transport of VDR is hampered do not differentiate when treated with 1,25D [15]. AML-associated chromosome translocation products, such as promyelocytic leukaemia (PML)/retinoic acid receptor  $\alpha$  (RAR $\alpha$ ), promyelocytic leukaemia zinc finger protein (PLZF)/RAR $\alpha$ , and AML-1/eight-twenty one protein (ETO), may block transport of VDR to the nucleus and reduce the capacity of AML cells to differentiate in response to 1,25D [16].

However, activation of VDR's transcriptional activity is not sufficient to explain all of the actions of 1,25D against AML cells. Hours are required for 1,25D to result in VDR-mediated transcription of gene products and some of the biological effects of 1,25D observed within AML cells happen in minutes. As to immediate effects in AML cells, 1,25D activates protein kinase C (PKC) [17], the extracellular-signal activated kinases (Erk) 1 and 2 [18] and the phosphatidylinositol 3-kinase (PI3K) [19]. Additionally, 1,25D activates lipid signalling pathways in AML cells. These are important to 1,25D-driven cell differentiation as it has been shown that inhibition of phospholipase D (PLD) blocks 1,25D-induced AML cells differentiation [20]. Enzymatic activity of PLD leads to the breakdown of membrane phospholipids, such as phosphatidylcholine and produces a second messenger, diacylglycerol (DAG). DAG in turn is an activator of the classical isoforms of PKC, which have been shown to be rapidly activated by 1,25D [17] and are on the pathway leading to activation of Erk1,2 [18]. Another lipid signalling enzyme that is activated in AML cells in response to 1,25D is phospholipase A<sub>2</sub> (PLA<sub>2</sub>) [21]. Activation of PLA<sub>2</sub> starts as early as 3 hours and lasts at least for 48h post-exposure of cells to 1,25D. Experiments using inhibitors of PLA<sub>2</sub> have shown that 1,25D-induced differentiation is enhanced when the activity of PLA<sub>2</sub> is inhibited. These experiments suggested that the end-products of the PLA<sub>2</sub> metabolic pathway provide a negative feedback to the action of 1,25D [22,23].

The mechanism whereby immediate signal events are provoked is still not entirely clear [24] as a number of mechanisms have been proposed to explain this phenomenon. One hypothesis is that a small portion of classical VDR is localized at the plasma membrane and 1,25D binds to an alternative ligand binding pocket to activate signal transduction from the cell membrane [25]. An alternative viewpoint is the existence of a membrane receptor for 1,25D that is distinct from VDR and is responsible for the rapid actions of 1,25D. Such a receptor has been shown to be expressed by intestine cells and is termed Membrane Associated Rapid Response Steroid-binding (MARRS) protein, which is also known as ERp57 or protein disulfide-isomerase A3 (PDIA3) [26,27]. Whether this receptor is present in AML cells has not been determined.

A primary feature of AML cells is their uncontrolled proliferation. In patients, the leukemic blast cells outnumber other blood cells. Moreover, the cells accumulating are non-functional because of a block at an early stage of myeloid cell differentiation. The use of 1,25D to inhibit the proliferation of AML cells and drive differentiation of these cells towards macrophages would be beneficial to patients or, at least, make their disease bearable. Additionally, AML patients are deficient in innate immune responsiveness and are prone to infection; restoration of some macrophage-related immunity might be beneficial. Unfortunately, there is a major drawback to the use of 1,25D as an anticancer agent and achievement of an effective therapeutic dose. The primary role of 1,25D is to regulate calcium and phosphate homeostasis, and when 1,25D is administered to patients at a high dose there are undesirable calcaemic effects. Serious side effects arising from hypercalcaemia include coma and cardiac arrest. The intermittent use of a high dose of 1,25D in patients with

advanced prostate cancer has been reported to be safe. However, the addition of 1,25D to dexamethasone treatment did not significantly improve the response rate [28]. The principle strategy to overcoming the problem of calcaemic effects has been the development of analogues of 1,25D with the desired anticancer effect without being calcaemic. Numerous analogues of 1,25D that have negligible calcaemic action have been synthesized [29,30]. Interestingly, neither the calcaemic nor the anti-proliferative potentials of analogues of 1,25D can be correlated directly to their affinities to VDR [30].

### 3. The actions of 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> against carcinomas

The hypothesis that vitamin D deficiency is a risk factor in terms of the development of prostate cancer was proposed in 1990 [31]. Schwartz and Hulka proposed that an appropriate level of vitamin D is essential to maintain the normal phenotype of prostate cells. Soon after, investigators documented the presence of VDR in prostate cells [32]. Later, epidemiological studies showed that serum levels of 25-hydroxyvitamin D<sub>3</sub> (25D) adversely correlate with prostate cancer risk [33]. This was most obvious in men living at a high latitude, for example, in Scandinavia, whose 25D level in blood serum was very low, below 16 ng/ml [34]. Numerous publications followed which documented that 1,25D has a beneficial action against prostate cancer cells. 1,25D was observed to inhibit proliferation, stimulate apoptosis, and interestingly have an anti-inflammatory effect towards VDR-positive prostate cancer cells [35]. These influences were accompanied by a decrease in the capacity of prostate cancer cells to migrate and, accordingly, their invasive potential [36,37]. A most surprising observation was that prostate cells have the capacity to convert biologically inert 25D into biologically active 1,25D. Previously, the only organ that was thought to be able to hydroxylate 25D to 1,25D was the kidney. In 1998 it was shown that normal prostate cells also possess the 1- $\alpha$ -hydroxylase to 25D (CYP27B1), which provides prostate cells with the means to synthesize 1,25D [38]. The activity of CYP27B1 has been examined in relation to the progressive steps of prostatic neoplasia, and the activity of CYP27B1 diminishes as cells progress from a normal phenotype, through hyperplasia to a malignant phenotype [39]. Other studies have provided data to further support the notion that a correct level of vitamin D and its autocrine conversion to 1,25D in the prostate are protective factors in prostate neoplasia. A high dietary intake of calcium has also been found to correlate with prostate cancer incidence [40] and this has substantially complicated the picture as a high level of circulating 1,25D increases the uptake of calcium from the intestine. An understanding of the role of 1,25D in prostate neoplasia is as yet incomplete but that 1,25D plays an important role in the development of prostate and prostate cancer is not questioned.

A large proportion of breast cancer cells contain the VDR protein, however the level of expression is variable within individual cells [41] and, therefore, the biological response to 1,25D varies for an individual patient's cells and between patients. The primary effect of exposure of breast cancer cells to 1,25D is cell cycle arrest due to changes in the functional status of the proteins that regulate cell cycle, such as retinoblastoma protein (Rb) and cyclin-dependent kinases and their inhibitors [42]. The induction of apoptosis is mediated by a reduction in the level of bcl-2 and up-regulation of the level of p53 [43]. An effect of 1,25D on cell proliferation is also mediated in a secondary way, via interference to the function of estrogen receptors (ER). 1,25D and its analogues down-modulate the expression of ER $\alpha$ , which in turn reduces the level of mitogenic signals to breast cancer cells from estrogens [44]. Another mechanism of the anticancer action of 1,25D against breast cancer cells is that it down-regulates the expression of aromatase, which catalyses a step in estrogen synthesis [45]. One of the key factors in regard to the activity of 1,25D is its availability in breast cancer environment. This is maintained by the balance between synthesis and catabolism. CYP27B1 is present in some breast cancer cells, to control the autocrine synthesis of 1,25D, but this enzyme is also active in breast cancer microenvironment. It has been shown that breast adipocytes produce CYP27B1, bio-activate 25D to 1,25D and in a paracrine fashion deliver 1,25D to the breast epithelium [46]. On the other hand, the availability of 1,25D is maintained by its degradation. This process is maintained by the 24-hydroxylase of 1,25D (CYP24A1). In normal tissues this enzyme is expressed in response to 1,25D exposure, providing a regulatory mechanism that maintains the concentration of

1,25D at a desired level [47]. Genome hybridization studies have revealed that in certain human breast cancers the *CYP24A1* gene is amplified and this may cause a reduction in the level of 1,25D and cells to proliferate unduly [48].

As mentioned above, an inverse correlation between the incidence malignancy and the level of vitamin D was first observed for colon cancer [2]. *In vitro* studies have shown that 1,25D and its analogues are able to inhibit the proliferation of colon cancer cells [49]. The anti-proliferative effect of 1,25D is revealed by an accumulation of cells in the G0/G1 phase of the cell cycle, and this is caused by an enhanced expression of cyclin-dependent kinase inhibitors p21<sup>CIP1</sup> and p27<sup>KIP1</sup> and reduced expression of cyclin A and cyclin F [50]. In addition, 1,25D sensitizes colon cancer cells to pro-apoptotic signals, through up-regulation of the pro-apoptotic and the down-regulation of the anti-apoptotic proteins [50]. Many studies have revealed that 1,25D promotes differentiation of colon cancer cells. This has been measured in terms of an expression of brush border enzymes and adhesion molecules, which confer the proper structure of colon epithelium, and acquisition of phenotypic features such as the presence of microvilli and cells having a polarized structure [50]. As for breast cancer, the vitamin D metabolic pathways appear to be important to colon tumorigenesis. *CYP27B1* expression is enhanced in high- to medium-differentiated human colon tumours as compared to tumour-adjacent normal mucosa or colon mucosa from non-cancer patients. In high-grade undifferentiated tumour areas expression of *CYP27B1* is decreased [51]. In contrast and in the majority of colon adenocarcinomas, expression of the 1,25D degrading enzyme *CYP24A1* is enhanced [52]. Moreover, the *CYP24A1* gene locus has been shown to be amplified in the majority of colorectal cancers and this gene has been proposed to be an oncogene [53].

#### 4. 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> and anticancer immunity

1,25D may be used as an anticancer agent alone and in combination with chemotherapeutic drugs as a means of enhancing the potency of such. As to the latter use, patients receiving chemotherapy are highly vulnerable to infections which can lead to death. Therefore it is important to ensure that 1,25D and new analogues are beneficial to good health and particularly do not have a damaging effect on the patient's immune system. In essence, an appropriate level of 1,25D is essential for good health and as to the correct function of the innate and adaptive immune systems.

The ancient Greeks first observed the benefit of exposure to sunlight, and presumably the synthesis of 1,25D, to good health. They used heliotherapy to treat the disease we now know as tuberculosis (TB). In 1903 Niels Finsen won the Nobel Prize for showing that exposure to ultra-violet light could be used to treat cutaneous TB. The need to promote public awareness of the importance of vitamin D to good health stems from knowledge of the consequences of vitamin D deficiency. A low systemic level of 1,25D leads to a higher risk of chronic diseases that affect a variety of organs. Such include diabetes [54], hypertension [55], inflammatory bowel disease [56] and kidney disease with a poor outcome at late stage [57]. Vitamin D also protects against osteoporosis [58], delays the onset of type 1- and 2-diabetes [54] and lowers the blood pressure in hypertensive animals [59]. There is the possibility that the association studies are revealing that 25D is a marker of poor health/lifestyle in general. Even so, cod liver oil has been used for over 100 years as an excellent source of vitamin D and a cure-all supplement, and the American Institute of Medicine recommends adequate intake (from UV, diet or supplement) of vitamin D at 600 IU per day up to the age of 70 and 800 IU if older [60].

1,25D enhances the protective effects of the innate immune response. Individuals who are vitamin D deficient [61] and those with inactivating mutations in the gene encoding VDR show defects in responses towards infectious agents (Etzioni et al., *Pediatric Res.* 1989, 25: 276-279). Tiosano and colleagues examined the immune status of fifteen hereditary vitamin-D resistant patients that have a truncated VDR which is unable to bind 1,25D and devoid of function. They reported no increased incidence of infectious, or autoimmune, diseases, but there were some differences between people with wild type and mutated VDR as to the capacity to mount immune responses. [62]. In keeping, it has been suggested that supplementation of vitamin D can help to fight off viral and other infections by boosting the function of the immune system [63,64]. Most of

the cells of the immune system express VDR and these cells also have the enzymes to make 1,25D from circulating 25D. As such, locally produced 1,25D plays an important role in enhancing the status of innate immunity. Blood-derived macrophages express VDR and can make their own 1,25D [65]. 1,25D promotes the proliferation and maturation of monocytes and macrophages and the migration of these cells to sites of infection. At sites of infection, the recognition of antigens by macrophage pattern recognition receptors switches on the production of 1,25D which, in turn, stimulates phagocytosis and the expression of the antimicrobial peptides defensin  $\beta$ 2 and cathelicidin gene product, to increase microbial killing (reviewed in [66]). However, production of AMP may not be the only mechanism by which 1,25D controls infection. In a murine model of pulmonary TB, bacterial load was unaffected, but granuloma size was increased and interferon  $\gamma$  (IFN $\gamma$ ) and tumour necrosis factor (TNF) producing CD4 cells decreased in animals on a high vitamin D diet. The reduction in immunopathology was protective against the pathogen and to the lung tissue [67]. Antimicrobial peptides such as cathelicidin have been implicated in both inducing, via receptors such as FPR2 and cell proliferation, and preventing cancer via direct binding to negatively charged membranes of tumour cells and induction of apoptosis [68,69].

1,25D may be of particular benefit to the immune status of older patients with cancer. With age, the immune system senesces and shifts towards an inflammatory profile, termed inflammaging. The chronic production of inflammatory cytokines appears to remodel the immune system [70,71]. Consequently, auto-immune diseases, such as rheumatoid arthritis, are more prevalent in older people [72]. In these disorders B lymphocytes make auto-antibodies with the help of T lymphocytes. 1,25D can dampen down the activity of the adaptive immune system. For example, *in vitro* studies have shown that 1,25D inhibits the generation of plasma cells, their expression of co-stimulatory molecules and the function of regulatory B cells. However, administration of a high dose of vitamin D to patients with multiple sclerosis has been shown to not have a substantial effect on B cell differentiation, isotype switching or the level of B cell activating factor [73]. Differences between findings from *in vitro* and *in vivo* studies indicate a more complex scenario and have led to speculation that the role of germinal centres in plasma cell production and/or other vitamins and hormones interfere with vitamin D-driven effects on B cells (reviewed in [74]). Further work is warranted to unravel the subtlety of the influence of 1,25D on B cell compartments.

Resting T lymphocytes do not express VDR but do so after they are activated. 1,25D has been shown to inhibit T lymphocyte proliferation and T helper (Th) cells that make interleukin (IL)-17 (Th 17 cells) and interferon- $\gamma$  (Th 1 cells). Th 1 and Th 17 cells can mediate immune diseases and these diseases are ameliorated by treatment with 1,25D. Th 1 and Th 17 responses are also important to host resistance to infectious diseases; 1,25D does not affect these responses. These findings lead to the viewpoint that 1,25D is a late regulator of the function of T lymphocytes and this function is important when there is chronic activation of T lymphocytes (reviewed in [75]). As to switching off/controlling immune responses, 1,25D helps to maintain self-tolerance by skewing the maturation of CD4 T lymphocytes away from a Th1 phenotype towards a tolerogenic Th2 or regulatory T cell phenotype. In this case, secretion of interferon- $\gamma$ , IL-17, IL-21 and IL-22 is suppressed and expression of forkhead box P3 (FOXP3), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and IL-10 is enhanced. Importantly, a low-calcaemic analogue of 1,25D has been used to treat T cells from healthy individuals to promote a stable regulatory profile [76]. Dendritic cells, through their interaction with T lymphocytes, are responsible for the generation of adaptive immunity and also have a tolerogenic role. The presence of 1,25D leads to dendritic cells acquiring tolerogenic properties [77]. The maturation of these cells is repressed by 1,25D as to reduced expression of the major histocompatibility complex and the costimulatory molecules CD40, CD80 and CD86. Secretion of IL-12 is decreased and IL-10 expression is enhanced which contributes to dendritic cells becoming tolerogenic in nature (reviewed in [66,78]). The inhibition of T cell priming by dendritic cells by 1,25D may be more complex than currently considered. On activation, dendritic cells express a truncated *CYP27B1* transcript that inhibits the conversion of 25D to active 1,25D, while *CYP24A1* levels were unaffected, leading to reduced autocrine 1,25D. Activation of vitamin D to 1,25D by macrophages has no such restraint. The production of 1,25D by macrophages induces expression of

VDR- responsive genes by dendritic cells and inhibits antigen stimulation-driven maturation of dendritic cells and dendritic cell-dependent T cell responses.. These data reveal that autocrine and paracrine activation of vitamin D are important to the control of dendritic cell function. [79]. Recent evidence shows that 1,25D may not directly inhibit the generation of antigen-specific CD4 T cells. In animals with experimental autoimmune encephalitis, myelin specific CD4 cells in the blood numbers were not affected, but treated animals had significantly decreased disease scores. Such antigen-specific cells left the lymph nodes and were present in the circulation but they did not enter the brain parenchyma. This was shown to be due to down-regulation of CXCR3, a receptor required for entry into the central nervous system (CNS) tissue [80].

Studies of VDR knock-out mice have brought to attention that responsiveness of cytotoxic T lymphocytes (CTLs) might be somehow affected by the absence of signals from 1,25D and VDR. These mice were able to clear *Listeria monocytogenes* infection, but slower than wild type counterparts, and their CTLs produced less IFN $\gamma$  [81]. These observations have focused attention on the role of 1,25D and VDR in CTL differentiation and cytokine production. A clear picture has still to emerge though the link between 1,25D and the incidence and/or severity of CTL-controlled infections is not disputable[61].

As stated above, low levels of 25D or 1,25D have been associated with disease in many autoimmune conditions. However, supplementation studies to date have proved inconclusive as to the prevention or control of autoimmune conditions, but this may be due to the dose and type of vitamin D used in the various studies [82]. A prospective randomised control trial with high dose vitamin D (up to 40,000 IU daily till 28 weeks, then 10,000IU daily for 12 weeks) in patients with multiple sclerosis was safe and reduced the relapse rate at 52 weeks [83]. More such randomised control trials with increased doses are needed to confirm these findings.

Many of the above findings as to effects of 1,25D on immune cells indicate the potential of 1,25D to restore immune tolerance and prevent the progression of autoimmune disease. The immune system of elderly patients with cancer might well be strengthened by the use of 1,25D to enhance the maintenance of tolerance, leading to the avoidance of autoimmune disorders. A conceivable down-side to the use of 1,25D to treat patients with cancer is whether any existing immunity to the tumour is switched from responsiveness towards a state of tolerance of the tumour cells. The extent to which this might occur is unknown and a benefit is most likely as to the anticancer action of 1,25D.

## 5. Clinical trials of the use of 1 $\alpha$ ,25-dihydroxyvitamin D $_3$ in cancer

### 5.1. Myelodysplasia (MDS) and AML

The results from *in vitro* studies of the activity of 1,25D against AML cell lines [6], patient-derived AML blasts [84,85] and pre-clinical tests in mice [86,87] clearly encouraged clinical trials. However, neither vitamin D nor its partially or fully active metabolites have proved to be very effective in clinical trials. One of the reasons for this failure was the clinical trials were performed on small groups of patients, ranging from 1 to 53, and the groups consisted of just MDS or AML patients, or patients with both conditions were included [88-97]. Moreover, the vitamin D compounds used in the clinical trials varied, as some used 1,25D whilst other trials made use of the 1,25D precursor 1 $\alpha$ -hydroxyvitamin D (1D, alfacalcidol). The doses used also varied, as well as the additions of other agents used in the treatments. The best results were observed for combination treatments. When MDS patients were treated with 1,25D and 13-cis-retinoic acid, the response rate was 52% and the transfusion need was reduced. However, the median survival time was not prolonged in these patients [96]. In a study which used 1,25D in combination with cytarabine and 13-cis-retinoic acid a response, to variable extent, was observed in 58% of patients [97]. Even better results were obtained using a combination of 1,25D with cytarabine and hydroxyurea: complete or partial responses were observed in 79% of elderly patients with AML [95].

### 5.2. Prostate cancer

There were several early clinical trials of the use of vitamin D compounds to treat prostate cancer. A pilot study revealed that administration of 2,000 IU of vitamin D daily led to a statistically significant delay in the rate of prostate specific antigen (PSA) rise in post-operative patients [98]. Another bigger study, conducted on 66 patients, confirmed the above results, and supported continuation of clinical trials [99]. In the later clinical study, performed on patients with advanced prostate cancer, 1,25D, the active form of vitamin D, was given together with dexamethasone [28]. Unfortunately, the response rate to this combination treatment was not seen to be significantly higher than when dexamethasone was given alone. However, high-dose intermittent 1,25D plus dexamethasone appeared to be safe for patients. The above, and some other encouraging results, have led to the development of a formulation of oral high dose 1,25D, named DN-101, by Novacea Inc. (San Francisco, CA). This new formulation in combination with docetaxel versus placebo and docetaxel was tested in a large, double-blinded, randomized study named ASCENT-1 [100]. The primary end-point, which was a PSA response within 6 months of enrolment, was not significantly different as to the DN-101 versus placebo arm. However, the median survival, which was not a primary endpoint in the trial, was estimated at 24.5 months in the DN-101 arm versus 16.4 months for placebo. This large survival advantage led to the next clinical trial, named ASCENT-2 [101]. Unfortunately, ASCENT-2 was terminated by Novacea Inc. on November 05, 2007, because of an unexpected and unexplained increased death rate in DN-101 arm.

Caution is warranted with respect to the use of vitamin D compounds to treat prostate cancer as the overall role of 1,25D in aggressive prostate cancer, in particular as to anti-inflammatory effects and disease pathogenesis and progression, is not well explored (Batai et al., *Front Immunol* 2016, 7:53). Additionally, it has been shown that men with high blood levels of 25D are at increased risk of developing prostate cancer (Albanes et al., *Cancer Epidemiol Biomarkers Prev* 2011, 20: 1850-1860).

### 5.3. Breast cancer

Many *in vitro* and pre-clinical studies have examined the possible use of 1,25D to treat patients with breast cancer. Four meta-analyses identified a significant inverse relationship between the circulating concentrations of 25D and breast cancer [102-105]. By contrast, the large randomised clinical trial WHI showed that administering 400 IU vitamin D and 1000 mg of calcium versus placebo to women did not reduce the risk of breast cancer [106]. The finding from Women's Health Initiative (WHI) does not provide support to the use of vitamin D as a prophylactic agent in the case of breast cancer. However, better survival among woman diagnosed with breast cancer has been reported to be related to vitamin D intake leading to higher concentrations of 25D [105,107]. Moreover, an interesting finding emerged from a retrospective review of patients with HER2<sup>ve</sup> breast cancer who had received chemotherapy with trastuzumab with or without vitamin D supplement (10,472 IU/week). Those who had taken the vitamin D supplements experienced significantly improved disease-free survival [108]. The conduct of a randomised, double-blind, placebo-controlled trial of vitamin D, or its active hormone 1,25D, with chemotherapy or other agents, is clearly important to revealing the benefit or otherwise of vitamin D/1,25D to treating breast cancer patients.

### 5.4. Colorectal cancer

Colorectal cancer has been a particular area of focus as to epidemiological studies of the role of vitamin D in cancer (reviewed in [109]) and pre-clinical studies of the use of 1,25D. As to studies of patients with colorectal cancer, there is a high prevalence of vitamin D deficiency among patients with stage IV colorectal cancer [110] and other studies support the notions that the formation of new adenomas appears not to be inhibited by vitamin D though progression through the carcinogenesis pathway and the growth of lesions are influenced by vitamin D [111]. A trial of vitamin D and calcium in patients who had had their colorectal adenomas removed did not reduce the risk, over a period of 3 to 5 years, of adenomas recurring [112]. However, whether vitamin D and calcium can prevent the recurrence of colorectal adenoma is still under investigation in the large, double-blind and randomised clinical trial The Vitamin D/Calcium Polyp Prevention Study [113]. The pending



results should provide further insight to whether vitamin D can reduce the incidence of adenoma recurrence. Hence, there isn't definitive evidence from patient studies to support vitamin D/1,25D as a reliable means to prevent and/or treat colorectal cancer. As brought to attention in a recent article, data obtained from studies of colorectal cancer provides the most compelling evidence as to a beneficial relationship between intake of vitamin D, and the level of serum 25D, and cancer, but "*the promise not yet fulfilled*" [114]. However, it is important to bear in mind there has been much discussion between vitamin D experts on what is a correct level of intake (Brouwer-Broekma et al., 2012, Osteoporosis Int. 24: 1567-1577). Even so, there has been a long term interest in the use of vitamin D/1,25D to treat colorectal cancer and more controlled and randomised trials are needed to resolve this matter.

### 5.5. Melanoma

Eighty percent of the daily requirement of vitamin D is made by the skin depending on appropriate exposure to sunlight. Melanoma is the most dangerous type of skin cancer and a low serum concentration of 25D is associated with both an increased risk of melanoma and unfavourable disease prognosis [115]. Studies have suggested a role for vitamin D in delaying the recurrence of melanoma and there are few treatment options for patients with primary cutaneous melanomas that are ulcerated and patients with nodal micro-metastases. ANZMTG (Australia and New Zealand Melanoma Trials Group) 02.09 Mel-D is an ongoing placebo controlled randomised phase II trial that is examining the safety and toxicity of an initial oral and large dose of vitamin D (500,000 IU) followed by a monthly oral dose of 50,000 IU for 2 years in patients who have had surgery to excise their primary cutaneous melanoma [116]. The findings from this study will be of interest as to the possible use of vitamin D, given orally, to treat another hard-to-treat cancer.

## 6. The extent to which low calcaemic analogues are in use

Recently, the result of a phase I multicenter trial in metastatic prostate cancer patients which combined the 1,25D analogue inecalcitol with docetaxel-based chemotherapy has been published. Inecalcitol is an analogue of 1,25D that shows agonistic activity towards VDR and was well tolerated in preclinical studies in mice [117]. The phase I study documented that inecalcitol was well tolerated and that 85% of patients had more than a 30% decline in PSA level within 3 months, while 76% of the patients had more than a 50% PSA decline at any time during the study [118].

Another analogue of 1,25D is paricalcitol which is related in chemical structure to vitamin D<sub>2</sub>. This analogue was tested in women receiving chemotherapy for metastatic breast cancer and the study documented that paricalcitol in combination with taxanes is safe and feasible [119]. This opens the possibility of larger clinical trials to assess health benefits of this analogue in women with breast cancer.

## 7. Conclusion

We can conclude there hasn't been substantial benefit to date as to the use of 1,25D to treat cancers. The results of trials conducted so far show that neither 1,25D nor analogues are sufficient anti-cancer agents when used alone. In essence, response rates have been insufficient and variable and there haven't been any dramatic increases in survival rates. *In vitro* and preclinical studies support the viewpoint that the various types of cancer are somewhat equally susceptible to the effects of 1,25D [120], and epidemiological studies clearly indicate that a correct level of serum vitamin D correlates with a low cancer incidence [109]. Careful analysis of these results may indicate that vitamin D is one of a few factors that correlate with the prevention of carcinogenesis. However, supplementing one of the factors, just vitamin D, may be not enough to slow down or cure the disease. Therefore, studies that aim to find accompanying factors, which act in concert with vitamin D, are necessary. Another possibility is that a low level of vitamin D or 25D is not causative for the above mentioned diseases. It should be also considered that such is a marker of poor health or a bad lifestyle. As yet, the verdict is uncertain in regard to the use of 1,25D analogues as an anticancer

agent particularly as the new analogues with negligible calcaemic action offer exciting prospects. These offer the possibility of achieving a more effective therapeutic dose with minimal side effects. When used in combination treatments they may deliver enhancement of the activities of other anti-cancer drugs or health benefits from their immune-modulatory actions.

**Acknowledgments:** This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no 315902. GB and EM are partners within the Marie Curie Initial Training Network DECIDE (Decision-making within cells and differentiation entity therapies).

**Conflicts of Interest:** The authors declare no conflict of interest.

## Abbreviations

The following abbreviations are used in this manuscript:

1,25D; 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub>;

25D; 25-hydroxyvitamin D<sub>3</sub>;

AML; acute myeloid leukemia;

CD; [cluster of differentiation](#);

CNS; central nervous system;

CTL, cytotoxic T lymphocyte;

CTLA-4; cytotoxic T-lymphocyte-associated protein 4;

CYP24A1; 24-hydroxylase of 1,25D;

CYP27B1; 1- $\alpha$ -hydroxylase to 25D;

DAG; diacylglycerol;

ER; estrogen receptors;

Erk; extracellular-signal activated kinase;

ETO; eight-twenty one protein;

FOXP3; forkhead box P3;

IL; interleukin;

IFN $\gamma$ ; interferon  $\gamma$ ;

MARRS; Membrane Associated Rapid Response Steroid-binding;

MDS; Myelodysplasia;

PDIA3; protein disulfide-isomerase A3;

PI3K; phosphatidyl inositol 3-kinase;

PKC; protein kinase C;

PLA2; phospholipase A<sub>2</sub>;

PLD; phospholipase D;

PLZF; promyelocytic leukaemia zinc finger protein;

PML; promyelocytic leukaemia;

PSA; prostate specific antigen;

RAR $\alpha$ ; retinoic acid receptor  $\alpha$ ;

Rb; retinoblastoma protein;

Th; T helper;

TNF; tumour necrosis factor;

VDR; vitamin D receptor;

WHI; Women's Health Initiative;

## References

1. Apperly, F. The relation of solar radiation to cancer mortality in north america. *Cancer Res* **1941**, *1*, 191-195.
2. Garland, C.; Garland, F. Do sunlight and vitamin d reduce the likelihood of colon cancer? *Int J Epidemiol* **1980**, *9*, 227-231.
3. Hanchette, C.; Schwartz, G. Geographic patterns of prostate cancer mortality. Evidence for a protective effect of ultraviolet radiation. *Cancer* **1992**, *70*, 2861-2869.
4. Garland, F.; Garland, C.; Gorham, E.; Young, J. Geographic variation in breast cancer mortality in the united states: A hypothesis involving exposure to solar radiation. *Prev. Med* **1990**, *19*, 614-622.
5. Abe, E.; Miamura, C.; Sakagami, H.; Takeda, M.; Konno, K.; Yamazaki, T.; Yoshiki, S.; Suda, T. Differentiation of mouse myeloid leukemia cells induced by 1-alpha,25-dihydroxyvitamin d<sub>3</sub>. *Proc Natl Acad Sci USA* **1981**, *78*, 4990-4994.
6. Miyaura, C.; Abe, E.; Kuribayashi, T.; Tanaka, H.; Konno, K.; Nishii, Y.; Suda, T. 1 alpha,25-dihydroxyvitamin d<sub>3</sub> induces differentiation of human myeloid leukemia cells. *Biochem Biophys Res Commun* **1981**, *102*, 937-943.
7. Godyn, J.; Xu, H.; Zhang, F.; Kolla, S.; Studzinski, G. A dual block to cell cycle progression in hl60 cells exposed to analogues of vitamin d<sub>3</sub>. *Cell Prolif* **1994**, *27*, 37-46.
8. Brown, G.; Choudhry, M.; Durham, J.; Drayson, M.; Michell, R. Monocytically differentiating hl60 cells proliferate rapidly before they mature. *Exp Cell Res* **1999**, *253*, 511-518.
9. Li, J.; Finch, R.; Sartorelli, A. Role of vitamin d<sub>3</sub> receptor in the synergistic differentiation of wehi-3b leukemia cells by vitamin d<sub>3</sub> and retinoic acid. *Exp Cell Res* **1999**, *249*, 279-290.
10. Gocek, E.; Marchwicka, A.; Burska, H.; Chrobak, A.; Marcinkowska, E. Opposite regulation of vitamin d receptor by atra in aml cells susceptible and resistant to vitamin d-induced differentiation. *J Steroid Biochem Mol Biol* **2012**, *132*, 220-226.
11. O'Kelly, J.; Histake, J.; Histake, Y.; Bishop, J.; Norman, A.; Koeffler, H. Normal myelopoiesis but abnormal t lymphocyte responses in vitamin d receptor knockout mice. *J Clin Invest* **2002**, *109*, 1091-1099.
12. Pike, J.; Meyer, M. Fundamentals of vitamin d hormone-regulated gene expression. *J Steroid Biochem Mol Biol* **2014**, Pt A, 5-11.
13. Carlberg, C.; Seuter, S.; de Mello, V.; Schwab, U.; Voutilainen, S.; Pulkki, K.; Nurmi, T.; Virtanen, J.; Tuomainen, T.; Uusitupa, M. Primary vitamin d target genes allow a categorization of possible benefits of vitamin d<sub>3</sub> supplementation. In *PLoS One* 2013; Vol. 8, p e71042.
14. Wang, Q.; Jones, J.; Studzinski, G. Cyclin-dependent kinase inhibitor p27 as a mediator of the g1-s phase block induced by 1,25-dihydroxyvitamin d<sub>3</sub> in hl60 cells. *Cancer Res* **1996**, *56*, 264-267.
15. Humeniuk-Polaczek, R.; Marcinkowska, E. Impaired nuclear localization of vitamin d receptor in leukemia cells resistant to calcitriol-induced differentiation. *J Steroid Biochem Mol Biol* **2004**, *88*, 361-366.

16. Puccetti, E.; Obradovic, D.; Beissert, T.; Bianchini, A.; Washburn, B.; Chiaradonna, F.; Boehrer, S.; Hoelzer, D.; Ottmann, O.; Pelicci, P., *et al.* Aml-associated translocation products block vitamin d<sub>3</sub>-induced differentiation by sequestering the vitamin d<sub>3</sub> receptor. *Cancer Res* **2002**, *62*, 7050-7058.
17. Berry, D.; Antochi, R.; Bhatia, M.; Meckling-Gill, K. 1,25-dihydroxyvitamin d<sub>3</sub> stimulates expression and translocation of protein kinase calpha and cdelta via a nongenomic mechanism and rapidly induces phosphorylation of a 33-kda protein in acute promyelocytic nb4 cells. *J Biol Chem* **1996**, *271*, 16090-16096.
18. Marcinkowska, E.; Wiedlocha, A.; Radzikowski, C. 1,25-dihydroxyvitamin d<sub>3</sub> induced activation and subsequent nuclear translocation of mapk is upstream regulated by pkc in hl-60 cells. *Biochem Biophys Res Commun* **1997**, *241*, 419-426.
19. Hmama, Z.; Nandan, D.; Sly, L.; Knutson, K.; Herrera-Velit, P.; Reiner, N. 1alpha,25-dihydroxyvitamin d(3)-induced myeloid cell differentiation is regulated by a vitamin d receptor-phosphatidylinositol 3-kinase signaling complex. *J Exp Med* **1999**, *190*, 1583-1594.
20. Hughes, P.; Brown, G. 1alpha,25-dihydroxyvitamin d<sub>3</sub>-mediated stimulation of steroid sulphatase activity in myeloid leukaemic cell lines requires vdrnuc-mediated activation of the ras/raf/erk-map kinase signalling pathway. *J Cell Biochem* **2006**, *98*, 590-617.
21. Aepfelbacher, F.; Weber, P.; Aepfelbacher, M. Activation of phospholipase a<sub>2</sub> by 1,25(oh)<sub>2</sub> vitamin d<sub>3</sub> and cell growth in monocytic u937 and mono mac 6 cells. *Cell Biochem Funct* **1995**, *13*, 19-23.
22. Marcinkowska, E.; Kutner, A. Side-chain modified vitamin d analogs require activation of both pi3-k and erk1,2 signal transduction pathways to induce differentiation of human promyelocytic leukemia cells. *Acta Biochim Pol* **2002**, *49*, 393-406.
23. López-Lluch, G.; Fernández-Ayala, D.; Alcaín, F.; Burón, M.; Quesada, J.; Navas, P. Inhibition of cox activity by nsaids or ascorbate increases camp levels and enhances differentiation in 1alpha,25-dihydroxyvitamin d<sub>3</sub>-induced hl-60 cells. *Arch Biochem Biophys* **2005**, *436*, 32-39.
24. Hughes, P.; Marcinkowska, E.; Gocek, E.; Studzinski, G.; Brown, G. Vitamin d<sub>3</sub>-driven signals for myeloid cell differentiation--implications for differentiation therapy. *Leuk Res* **2010**, *34*, 553-565.
25. Mizwicki, M.; Norman, A. Vitamin d sterol/vdr conformational dynamics and nongenomic actions. In *Vitamin d, 3rd edition*, Feldman, D.; Pike, J.; Adams, J., Eds. Academic Press: 2011; Vol. 1.
26. Sterling, T.; Khanal, R.; Nemere, I. The 1,25 dihydroxyvitamin d<sub>3</sub>-membrane-associated, rapid response steroid-binding receptor. *OA Biochemistry* **2013**, *1*, 4.
27. Nemere, I. The 1,25d<sub>3</sub>-marrs protein: Contribution to steroid stimulated calcium uptake in chicks and rats. *Steroids* **2005**, *70*, 455-457.
28. Trump, D.; Potter, D.; Muindi, J.; Brufsky, A.; Johnson, C. Phase ii trial of high-dose, intermittent calcitriol (1,25 dihydroxyvitamin d<sub>3</sub>) and dexamethasone in androgen-independent prostate cancer. *Cancer* **2006**, *106*, 2136-2142.
29. Bouillion, R.; Okamura, W.; Norman, A. Structure-function relationships in the vitamin d endocrine system. *Endocrine Reviews* **1995**, *16*, 200-216.
30. Nadkarni, S.; Chodyski, M.; Corcoran, A.; Marcinkowska, E.; Brown, G.; Kutner, A. Double point modified analogs of vitamin d as potent activators of vitamin d receptor. *Curr Pharm Des* **2015**, *21*, 1741-1763.
31. Schwartz, G.; Hulka, B. Is vitamin d deficiency a risk factor for prostate cancer? (hypothesis). *Anticancer Res* **1990**, *10*, 1307-1311.
32. Miller, G.; Stapleton, G.; Ferrara, J.; Lucia, M.; Pfister, S.; Hedlund, T.; Upadhy, P. The human prostatic carcinoma cell line Incap expresses biologically active, specific receptors for 1 alpha,25-dihydroxyvitamin d<sub>3</sub>. *Cancer Res* **1992**, *52*, 515-520.
33. Ahonen, M.; Tenkanen, L.; Teppo, L.; Hakama, M.; Tuohimaa, P. Prostate cancer risk and prediagnostic serum 25-hydroxyvitamin d levels (finland). *Cancer Causes Control* **2000**, *11*, 847-852.
34. Schwartz, G. Vitamin d deficiency and the epidemiology of prostate cancer. In *Vitamin d: Physiology, molecular biology, and clinical applications*, Holick, M., Ed. Humana Press: New York, 2010; pp 797-811.
35. Moreno, J.; Krishnan, A.; Feldman, D. Molecular mechanisms mediating the anti-proliferative effects of vitamin d in prostate cancer. *J Steroid Biochem Mol Biol* **2005**, *97*, 31-36.
36. Sung, V.; Feldman, D. 1,25-dihydroxyvitamin d<sub>3</sub> decreases human prostate cancer cell adhesion and migration. *Mol Cell Endocrinol* **2000**, *164*, 133-143.
37. Schwartz, G.; Wang, M.; Zang, M.; Singh, R.; Siegal, G. 1 alpha,25-dihydroxyvitamin d (calcitriol) inhibits the invasiveness of human prostate cancer cells. *Cancer Epidemiol Biomarkers Prev* **1997**, *6*, 727-732.

38. Young, M.; Schwartz, G.; Wang, L.; Jamieson, D.; Whitlatch, L.; Flanagan, J.; Lokeshwar, B.; Holick, M.; Chen, T. The prostate 25-hydroxyvitamin d-1 alpha-hydroxylase is not influenced by parathyroid hormone and calcium: Implications for prostate cancer chemoprevention by vitamin d. *Carcinogenesis* **2004**, *25*, 967-971.
39. Whitlatch, L.; Young, M.; Schwartz, G.; Flanagan, J.; Burnstein, K.; Lokeshwar, B.; Rich, E.; Holick, M.; Chen, T. 25-hydroxyvitamin d-1alpha-hydroxylase activity is diminished in human prostate cancer cells and is enhanced by gene transfer. *J Steroid Biochem Mol Biol* **2002**, *81*, 135-140.
40. Gao, X.; LaValley, M.; Tucker, K. Prospective studies of dairy product and calcium intakes and prostate cancer risk: A meta-analysis. *J Natl Cancer Inst* **2005**, *97*, 1768-1777.
41. Eisman, J.; Martin, T.; MacIntyre, I. Presence of 1,25-dihydroxy vitamin d receptor in normal and abnormal breast tissue. *Prog Biochem Pharmacol* **1980**, *17*, 143-150.
42. Simboli-Campbell, M.; Narvaez, C.; van Weelden, K.; Tenniswood, M.; Welsh, J. Comparative effects of 1,25(OH)<sub>2</sub>D<sub>3</sub> and eb1089 on cell cycle kinetics and apoptosis in mcf-7 breast cancer cells. *Breast Cancer Res Treat* **1997**, *42*, 31-41.
43. James, S.; Mackay, A.; Colston, K. Effects of 1,25 dihydroxyvitamin d<sub>3</sub> and its analogues on induction of apoptosis in breast cancer cells. *J Steroid Biochem Mol Biol* **1996**, *58*, 395-401.
44. James, S.; Mackay, A.; Binderup, L.; Colston, K. Effects of a new synthetic vitamin d analogue, eb1089, on the oestrogen-responsive growth of human breast cancer cells. *J Endocrinol* **1994**, *141*, 555-563.
45. Krishnan, A.; Swami, S.; Peng, L.; Wang, J.; Moreno, J.; Feldman, D. Tissue-selective regulation of aromatase expression by calcitriol: Implications for breast cancer therapy. *Endocrinology* **2010**, *151*, 32-42.
46. Ching, S.; Kashinkunti, S.; Niehaus, M.; Zinser, G. Mammary adipocytes bioactivate 25-hydroxyvitamin d<sub>3</sub> and signal via vitamin d<sub>3</sub> receptor, modulating mammary epithelial cell growth. *J Cell Biochem* **2011**, *112*, 3393-3405.
47. Vaisanen, S.; Dunlop, T.; Sinkkonen, L.; Frank, C.; Carlberg, C. Spatio-temporal activation of chromatin on the human cyp24 gene promoter in the presence of 1alpha,25-dihydroxyvitamin d<sub>3</sub>. *J Mol Biol* **2005**, *350*, 65-77.
48. Albertson, D.; Ylstra, B.; Segraves, R.; Collins, C.; Dairkee, S.; Kowbel, D.; Kuo, W.; Gray, J.; Pinkel, D. Quantitative mapping of amplicon structure by array cgh identifies cyp24 as a candidate oncogene. *Nat Genet* **2000**, *25*, 144-146.
49. Cross, H.; Huber, C.; Peterlik, M. Antiproliferative effect of 1,25-dihydroxyvitamin d<sub>3</sub> and its analogs on human colon adenocarcinoma cells (caco-2): Influence of extracellular calcium. *Biochem Biophys Res Commun* **1991**, *179*, 57-62.
50. Pereira, F.; Larriba, M.; Muñoz, A. Vitamin d and colon cancer. *Endocr Relat Cancer* **2012**, *19*, R51-71.
51. Bises, G.; Kállay, E.; Weiland, T.; Wrba, F.; Wenzl, E.; Bonner, E.; Kriwanek, S.; Obrist, P.; Cross, H. 25-hydroxyvitamin d<sub>3</sub>-1alpha-hydroxylase expression in normal and malignant human colon. *J Histochem Cytochem* **2004**, *52*, 985-989.
52. Horváth, H.; Lakatos, P.; Kósa, J.; Bácsi, K.; Borka, K.; Bises, G.; Nittke, T.; Herschberger, P.; Speer, G.; Kállay, E. The candidate oncogene cyp24a1: A potential biomarker for colorectal tumorigenesis. *J Histochem Cytochem* **2010**, *58*, 277-285.
53. Höbaus, J.; Hummel, D.; Thiem, U.; Fetahu, I.; Aggarwal, A.; Müllauer, L.; Heller, G.; Egger, G.; Mesteri, I.; Baumgartner-Parzer, S., et al. Increased copy-number and not DNA hypomethylation causes overexpression of the candidate proto-oncogene cyp24a1 in colorectal cancer. *Int J Cancer* **2013**, *133*, 1380-1388.
54. Nwosu, B.; Maranda, L. The effects of vitamin d supplementation on hepatic dysfunction, vitamin d status, and glycemic control in children and adolescents with vitamin d deficiency and either type 1 or type 2 diabetes mellitus. *PLoS One* **2014**, *9*, 6.
55. Carbone, F.; Mach, F.; Vuilleumier, N.; Montecucco, F. Potential pathophysiological role for the vitamin d deficiency in essential hypertension. *World J Cardiol* **2014**, *6*, 260-276.
56. Ooi, J.; Chen, J.; Cantorna, M. Vitamin d regulation of immune function in the gut: Why do t cells have vitamin d receptors? *Mol Aspects Med* **2012**, *33*, 77-82.
57. Pilz, S.; Iodice, S.; Zittermann, A.; Grant, W.; Gandini, S. Vitamin d status and mortality risk in ckd: A meta-analysis of prospective studies. *Am J Kidney Dis* **2011**, *58*, 374-382.
58. Kim, S. The pleiomorphic actions of vitamin d and its importance for children. *Ann Pediatr Endocrinol Metab* **2013**, *18*, 45-54.

59. Vaidya, A.; Forman, J. Vitamin d and vascular disease: The current and future status of vitamin d therapy in hypertension and kidney disease. *Curr Hypertens Rep* **2012**, *14*, 111-119.
60. Boucher, B. The 2010 recommendations of the american institute of medicine for daily intakes of vitamin d. *Public Health Nutr* **2011**, *14*, 740.
61. Sarkar, S.; Hewison, M.; Studzinski, G.; Li, Y.; Kalia, V. Role of vitamin d in cytotoxic t lymphocyte immunity to pathogens and cancer. *Crit Rev Clin Lab Sci* **2016**, *53*, 132-145.
62. Tiosano, D.; Wildbaum, G.; Gepstein, V.; Verbitsky, O.; Weisman, Y.; Karin, N.; Ezzioni, A. The role of vitamin d receptor in innate and adaptive immunity: A study in hereditary vitamin d-resistant rickets patients. *J Clin Endocrinol Metab* **2013**, *98*, 1685-1693.
63. Chun, R.; Liu, P.; Modlin, R.; Adams, J.; Hewison, M. Impact of vitamin d on immune function: Lessons learned from genome-wide analysis. *Front Physiol* **2014**, *5*, 151.
64. Korf, H.; Decallonne, B.; Mathieu, C. Vitamin d for infections. *Curr Opin Endocrinol Diabetes Obes* **2014**, *21*, 431-436.
65. Hayes, M.; Bayley, D.; Drayson, M.; Freemont, A.; Denton, J.; Davies, M.; Mawer, E. Metabolism of 25-hydroxyvitamin d<sub>3</sub> to 24,25-dihydroxyvitamin d<sub>3</sub> by blood derived macrophages from a patient with alveolar rhabdomyosarcoma during short-term culture and 1 alpha,25-dihydroxyvitamin d<sub>3</sub> after long-term culture. *J Steroid Biochem Mol Biol* **1991**, *38*, 301-306.
66. Hewison, M. Vitamin d and immune function: Autocrine, paracrine or endocrine? *Scand J Clin Lab Invest Suppl* **2012**, *243*, 92-102.
67. Reeme, A.; Robinson, R. Dietary vitamin d<sub>3</sub> suppresses pulmonary immunopathology associated with late-stage tuberculosis in c3heb/fej mice. *J Immunol* **2016**, *196*, 1293-1304.
68. Weber, G.; Chamorro, C.; Granath, F.; Liljegren, A.; Zreika, S.; Saidak, Z.; Sandstedt, B.; Rotstein, S.; Mentaverri, R.; Sánchez, F., et al. Human antimicrobial protein hcgp18/IL-37 promotes a metastatic phenotype in breast cancer. *Breast Cancer Res* **2009**, *11*, R6.
69. Ren, S.; Shen, J.; Cheng, A.; Lu, L.; Chan, R.; Li, Z.; Wang, X.; Wong, C.; Zhang, L.; Ng, S., et al. Fk-16 derived from the anticancer peptide IL-37 induces caspase-independent apoptosis and autophagic cell death in colon cancer cells. *PLoS One* **2013**, *8*, e63641.
70. Franceschi, C. Inflammaging as a major characteristic of old people: Can it be prevented or cured? *Nutr Rev* **2007**, *65*, S173-176.
71. Minciullo, P.; Catalano, A.; Mandraffino, G.; Casciaro, M.; Crucitti, A.; Maltese, G.; Morabito, N.; Lasco, A.; Gangemi, S.; Basile, G. Inflammaging and anti-inflammaging: The role of cytokines in extreme longevity. *Arch Immunol Ther Exp (Warsz)* **2015**, [Epub ahead of print].
72. Jagger, A.; Shimojima, Y.; Goronzy, J.; Weyand, C. Regulatory t cells and the immune aging process: A mini-review. *Gerontology* **2014**, *60*, 130-137.
73. Knippenberg, S.; Smolders, J.; Thewissen, M.; Peelen, E.; Tervaert, J.; Hupperts, R.; Damoiseaux, J. Effect of vitamin d(3) supplementation on peripheral b cell differentiation and isotype switching in patients with multiple sclerosis. *Mult Scler* **2011**, *17*, 1418-1423.
74. Rolf, L.; Muris, A.; Hupperts, R.; Damoiseaux, J. Illuminating vitamin d effects on b-cells - the multiple sclerosis perspective. *Immunology* **2016**, [Epub ahead of print].
75. Cantorna, M.; Waddell, A. The vitamin d receptor turns off chronically activated t cells. *Ann N Y Acad Sci* **2014**, *1317*, 70-75.
76. Van Belle, T.; Vanherwegen, A.; Feyaerts, D.; De Clercq, P.; Verstuyf, A.; Korf, H.; Gysemans, C.; Mathieu, C. 1,25-dihydroxyvitamin d<sub>3</sub> and its analog tx527 promote a stable regulatory t cell phenotype in t cells from type 1 diabetes patients. *PLoS One* **2014**, *9*, e109194.
77. Ferreira, G.; Gysemans, C.; Demengeot, J.; da Cunha, J.; Vanherwegen, A.; Overbergh, L.; Van Belle, T.; Pauwels, F.; Verstuyf, A.; Korf, H., et al. 1,25-dihydroxyvitamin d<sub>3</sub> promotes tolerogenic dendritic cells with functional migratory properties in nod mice. *J Immunol* **2014**, *192*, 4210-4220.
78. Barragan, M.; Good, M.; Kolls, J. Regulation of dendritic cell function by vitamin d. *Nutrients* **2015**, *7*, 8127-8151.
79. Kundu, R.; Chain, B.; Coussens, A.; Khoo, B.; Noursadeghi, M. Regulation of cyp27b1 and cyp24a1 hydroxylases limits cell-autonomous activation of vitamin d in dendritic cells. *Eur J Immunol* **2014**, *44*, 1781-1790.
80. Grishkan, I.; Fairchild, A.; Calabresi, P.; Gocke, A. 1,25-dihydroxyvitamin d<sub>3</sub> selectively and reversibly impairs t helper-cell cns localization. *Proc Natl Acad Sci USA* **2013**, *110*, 21101-21106.
81. Bruce, D.; Whitcomb, J.; August, A.; McDowell, M.; Cantorna, M. Elevated non-specific immunity and normal listeria clearance in young and old vitamin d receptor knockout mice. *Int Immunol* **2009**, *21*, 113-122.

82. Antico, A.; Tampoia, M.; Tozzoli, R.; Bizzaro, N. Can supplementation with vitamin d reduce the risk or modify the course of autoimmune diseases? A systematic review of the literature. *Autoimmun Rev* **2012**, *12*, 127-136.
83. Burton, J.; Kimball, S.; Vieth, R.; Bar-Or, A.; Dosch, H.; Cheung, R.; Gagne, D.; D'Souza, C.; Ursell, M.; O'Connor, P. A phase i/ii dose-escalation trial of vitamin d<sub>3</sub> and calcium in multiple sclerosis. *Neurology* **2010**, *74*, 1852-1859.
84. Wang, Q.; Harrison, J.; Uskokovic, M.; Kutner, A.; Studzinski, G. Translational study of vitamin d differentiation therapy of myeloid leukemia: Effects of the combination with a p38 mapk inhibitor and an antioxidant. *Leukemia* **2005**, *19*, 1812-1817.
85. Gocek, E.; Kielbinski, M.; Bauriska, H.; Haus, O.; Kutner, A.; Marcinkowska, E. Different susceptibilities to 1,25-dihydroxyvitamin d<sub>3</sub>-induced differentiation of aml cells carrying various mutations. *Leuk Res* **2010**, *34*, 649-657.
86. Honma, Y.; Hozumi, M.; Abe, E.; Konno, K.; Fukushima, M.; Hata, S.; Nishii, Y.; DeLuca HF; Suda, T. 1-alpha,25-dihydroxyvitamin d<sub>3</sub> and 1-alpha-hydroxyvitamin d<sub>3</sub> prolong survival time of mice inoculated with myeloid leukemia cells. *Proc Natl Acad Sci U S A* **1983**, *80*, 201-204.
87. Shabtay, A.; Sharabani, H.; Barvish, Z.; Kafka, M.; Amichay, D.; Levy, J.; Sharoni, Y.; Uskokovic, M.; Studzinski, G.; Danilenko, M. Synergistic antileukemic activity of carnosic acid-rich rosemary extract and the 19-nor gemini vitamin d analogue in a mouse model of systemic acute myeloid leukemia. *Oncology* **2008**, *75*, 203-214.
88. Hellström, E.; Robèrt, K.; Gahrton, G.; Mellstedt, H.; Lindemalm, C.; Einhorn, S.; Björkholm, M.; Grimfors, G.; Udén, A.; Samuelsson, J. Therapeutic effects of low-dose cytosine arabinoside, alpha-interferon, 1 alpha-hydroxyvitamin d<sub>3</sub> and retinoic acid in acute leukemia and myelodysplastic syndromes. *Eur J Haematol* **1988**, *40*, 449-459.
89. Hellström, E.; Robèrt, K.; Samuelsson, J.; Lindemalm, C.; Grimfors, G.; Kimby, E.; Oberg, G.; Winqvist, I.; Billström, R.; Carneskog, J. Treatment of myelodysplastic syndromes with retinoic acid and 1 alpha-hydroxy-vitamin d<sub>3</sub> in combination with low-dose ara-c is not superior to ara-c alone. Results from a randomized study. The scandinavian myelodysplasia group (smg). *Eur J Haematol* **1990**, *45*, 255-261.
90. Irino, S.; Taoka, T. Treatment of myelodysplastic syndrome and acute myelogenous leukemia with vitamin d<sub>3</sub> [1 alpha(oh)d<sub>3</sub>]. *Gan To Kagaku Ryoho* **1988**, *15*, 1183-1190.
91. Nakayama, S.; Ishikawa, T.; Yabe, H.; Nagai, K.; Kasakura, S.; Uchino, H. Successful treatment of a patient with acute myeloid leukemia with 1 alpha(oh)d<sub>3</sub>. *Nihon Ketsueki Gakkai Zasshi* **1988**, *51*, 1026-1030.
92. Takahashi, T.; Ichiba, S.; Okuno, Y.; Sugiyama, H.; Sakai, Y.; Imura, H.; Iho, S.; Hoshino, T.; Suzuki, A.; Okada, T. Therapeutic effectiveness of vitamin d<sub>3</sub> in patients with myelodysplastic syndromes, leukemias and myeloproliferative disorders. *Rinsho Ketsueki* **1989**, *30*, 1-10.
93. Petrini, M.; Caracciolo, F.; Corini, M.; Valentini, P.; Sabbatini, A.; Grassi, B. Low-dose ara-c and 1(oh)d<sub>3</sub> administration in acute non lymphoid leukemia: Pilot study. *Haematologica* **1991**, *76*, 200-203.
94. Petrini, M.; Dastoli, G.; Valentini, P.; Mattii, L.; Trombi, L.; Testi, R.; Ambrogi, F.; Grassi, B. Synergistic effects of alpha interferon and 1,25 dihydroxyvitamin d<sub>3</sub>: Preliminary evidence suggesting that interferon induces expression of the vitamin receptor. *Haematologica* **1991**, *76*, 467-471.
95. Slapak, C.; Desforges, J.; Fogaren, T.; Miller, K. Treatment of acute myeloid leukemia in the elderly with low-dose cytarabine, hydroxyurea, and calcitriol. *Am J Hematol* **1992**, *41*, 178-183.
96. Ferrero, D.; Bruno, B.; Pregno, P.; Stefani, S.; Larizza, E.; Ciravegna, G.; Luraschi, A.; Vietti-Ramus, G.; Schinco, P.; Bazzan, M., et al. Combined differentiating therapy for myelodysplastic syndromes: A phase ii study. *Leuk Res* **1996**, *20*, 867-876.
97. Ferrero, D.; Campa, E.; Dellacasa, C.; Campana, S.; Foli, C.; Boccadoro, M. Differentiating agents + low-dose chemotherapy in the management of old/poor prognosis patients with acute myeloid leukemia or myelodysplastic syndrome. *Haematologica* **2004**, *89*, 619-620.
98. Woo, T.; Choo, R.; Jamieson, M.; Chander, S.; Vieth, R. Pilot study: Potential role of vitamin d (cholecalciferol) in patients with psa relapse after definitive therapy. *Nutr Cancer* **2005**, *51*, 32-36.
99. Wagner, D.; Trudel, D.; Van der Kwast, T.; Nonn, L.; Giangreco, A.; Li, D.; Dias, A.; Cardoza, M.; Laszlo, S.; Hersey, K., et al. Randomized clinical trial of vitamin d<sub>3</sub> doses on prostatic vitamin d metabolite levels and ki67 labeling in prostate cancer patients. *J Clin Endocrinol Metab* **2013**, *98*, 1498-1507.
100. Beer, T.; Ryan, C.; Venner, P.; Petrylak, D.; Chatta, G.; Ruether, J.; Redfern, C.; Fehrenbacher, L.; Saleh, M.; Waterhouse, D., et al. Double-blinded randomized study of high-dose calcitriol plus

- docetaxel compared with placebo plus docetaxel in androgen-independent prostate cancer: A report from the ascent investigators. *J Clin Oncol* **2007**, *25*, 669-674.
101. 101. Schwartz, G. Vitamin d and intervention trials in prostate cancer: From theory to therapy. *Ann Epidemiol* **2009**, *19*, 96-102.
  102. 102. Yin, L.; Grandi, N.; Raum, E.; Haug, U.; Arndt, V.; Brenner, H. Meta-analysis: Serum vitamin d and breast cancer risk. *Eur J Cancer* **2010**, *46*, 2196-2205.
  103. 103. Gandini, S.; Boniol, M.; Haukka, J.; Byrnes, G.; Cox, B.; Sneyd, M.; Mullie, P.; Autier, P. Meta-analysis of observational studies of serum 25-hydroxyvitamin d levels and colorectal, breast and prostate cancer and colorectal adenoma. *Int J Cancer* **2011**, *128*, 1414-1424.
  104. 104. Chen, P.; Li, M.; Gu, X.; Liu, Y.; Li, X.; Li, C.; Wang, Y.; Xie, D.; Wang, F.; Yu, C., *et al.* Higher blood 25(oh)d level may reduce the breast cancer risk: Evidence from a chinese population based case-control study and meta-analysis of the observational studies. *PLoS One* **2013**, *8*, e49312.
  105. 105. Kim, Y.; Je, Y. Vitamin d intake, blood 25(oh)d levels, and breast cancer risk or mortality: A meta-analysis. *Br J Cancer* **2014**, *110*, 2772-2784.
  106. 106. Chlebowski, R.; Johnson, K.; Kooperberg, C.; Pettinger, M.; Wactawski-Wende, J.; Rohan, T.; Rossouw, J.; Lane, D.; O'Sullivan, M.; Yasmeen, S., *et al.* Calcium plus vitamin d supplementation and the risk of breast cancer. *J Natl Cancer Inst* **2008**, *100*, 1581-1591.
  107. 107. Vrieling, A.; Seibold, P.; Johnson, T.; Heinz, J.; Obi, N.; Kaaks, R.; Flesch-Janys, D.; Chang-Claude, J. Circulating 25-hydroxyvitamin d and postmenopausal breast cancer survival: Influence of tumor characteristics and lifestyle factors? *Int J Cancer* **2014**, *134*, 2972-2983.
  108. 108. Zeichner, S.; Koru-Sengul, T.; Shah, N.; Liu, Q.; Markward, N.; Montero, A.; Glück, S.; Silva, O.; Ahn, E. Improved clinical outcomes associated with vitamin d supplementation during adjuvant chemotherapy in patients with her2+ nonmetastatic breast cancer. *Clin Breast Cancer* **2015**, *15*, e1-11.
  109. 109. Jacobs, E.; Kohler, L.; Kunihiro, A.; Jurutka, P. Vitamin d and colorectal, breast, and prostate cancers: A review of the epidemiological evidence. *J Cancer* **2016**, *7*, 232-240.
  110. 110. Ng, K.; Sargent, D.; Goldberg, R.; Meyerhardt, J.; Green, E.; Pitot, H.; Hollis, B.; Pollak, M.; Fuchs, C. Vitamin d status in patients with stage iv colorectal cancer: Findings from intergroup trial n9741. *J Clin Oncol* **2011**, *29*, 1599-1606.
  111. 111. Yin, L.; Grandi, N.; Raum, E.; Haug, U.; Arndt, V.; Brenner, H. Meta-analysis: Serum vitamin d and colorectal adenoma risk. *Prev Med* **2011**, *53*, 10-16.
  112. 112. Baron, J.; Barry, E.; Mott, L.; Rees, J.; Sandler, R.; Snover, D.; Bostick, R.; Ivanova, A.; Cole, B.; Ahnen, D., *et al.* A trial of calcium and vitamin d for the prevention of colorectal adenomas. *N Engl J Med* **2015**, *373*, 1519-1530.
  113. 113. Barry, E.; Rees, J.; Peacock, J.; Mott, L.; Amos, C.; Bostick, R.; Figueiredo, J.; Ahnen, D.; Bresalier, R.; Burke, C., *et al.* Genetic variants in cyp2r1, cyp24a1, and vdr modify the efficacy of vitamin d3 supplementation for increasing serum 25-hydroxyvitamin d levels in a randomized controlled trial. *J Clin Endocrinol Metab* **2014**, *99*, E2133-2137.
  114. 114. Bikle, D. Vitamin d and cancer: The promise not yet fulfilled. *Endocrine* **2014**, *46*, 29-38.
  115. 115. Bade, B.; Zdebik, A.; Wagenpfeil, S.; Gräber, S.; Geisel, J.; Vogt, T.; Reichrath, J. Low serum 25-hydroxyvitamin d concentrations are associated with increased risk for melanoma and unfavourable prognosis. *PLoS One* **2014**, *9*, e112863.
  116. 116. Saw, R.; Armstrong, B.; Mason, R.; Morton, R.; Shannon, K.; Spillane, A.; Stretch, J.; Thompson, J. Adjuvant therapy with high dose vitamin d following primary treatment of melanoma at high risk of recurrence: A placebo controlled randomised phase ii trial (anzmtg 02.09 mel-d). *BMC Cancer* **2014**, *14*, 780.
  117. 117. Okamoto, R.; Delansorne, R.; Wakimoto, N.; Doan, N.; Akagi, T.; Shen, M.; Ho, Q.; Said, J.; Koeffler, H. Inecalcitol, an analog of 1 $\alpha$ ,25(oh)(2) d(3) , induces growth arrest of androgen-dependent prostate cancer cells. *Int J Cancer* **2012**, *130*, 2464-2473.
  118. 118. Medioni, J.; Deplanque, G.; Ferrero, J.; Maurina, T.; Rodier, J.; Raymond, E.; Allyon, J.; Maruani, G.; Houillier, P.; Mackenzie, S., *et al.* Phase i safety and pharmacodynamic of inecalcitol, a novel vdr agonist with docetaxel in metastatic castration-resistant prostate cancer patients. *Clin Cancer Res* **2014**, *20*, 4471-4477.
  119. 119. Lawrence, J.; Akman, S.; Melin, S.; Case, L.; Schwartz, G. Oral paricalcitol (19-nor-1,25-dihydroxyvitamin d2) in women receiving chemotherapy for metastatic breast cancer: A feasibility trial. *Cancer Biol Ther* **2013**, *14*, 476-480.
  120. 120. Krishnan, A.; Trump, D.; Johnson, C.; Feldman, D. The role of vitamin d in cancer prevention and treatment. *Rheum Dis Clin North Am* **2012**, *38*, 161-178.
  - 121.



122.



© 2016 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons by Attribution (CC-BY) license (<http://creativecommons.org/licenses/by/4.0/>).